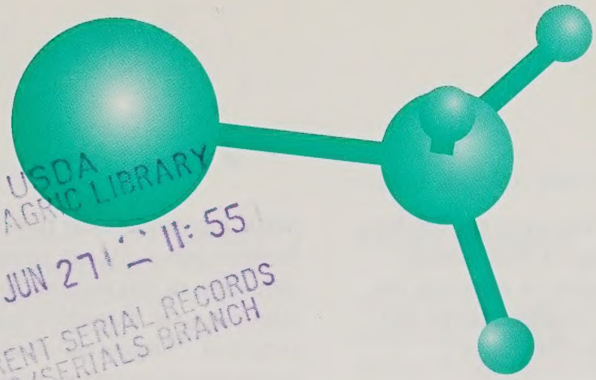
Methyl Bromide *Alternatives*



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Inside This Issue

This issue and all back issues of the *Methyl Bromide Alternatives* newsletter are now available on the Internet at **<http://www.ars.usda.gov/is/np/mba/mebrhp.htm>**.
Visit the ARS methyl bromide research homepage at **<http:/www.ars.usda.gov/ismbmebrweb.htm>**.

This newsletter provides information on research for methyl bromide alternatives from USDA, universities, and industry.

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Growers—Key to Research

Scientific research often creates images of test tubes and highly sophisticated equipment. In agricultural research, however, field studies also are necessary to determine the efficacy of a new pesticide, herbicide, or biological control agent. This is particularly true in the search for effective alternatives to methyl bromide.

The search for alternatives for methyl bromide has resulted in some viable options for growers. These advances would not have come this far without the willingness of growers to test the alternatives in their fields.

Research studies are effective tools in determining if an alternative treatment *may* work. The only way to determine if a treatment *will* work is to try it in the real world of a farm field. A treatment that works in a pot study or microplot may not be effective in a large-scale field trial.

"Most growers don't believe it's possible to extrapolate outcomes from pot studies, microplots, and small plots at universities," says Joseph Noling, an extension nematologist with the University of Florida's Institute of Food and Agricultural Sciences at Lake Alfred. "They understand they have a role to verify the findings of studies."

The relationship between grower and researcher is mutually beneficial. The researcher finds out if he or she is on the right track and the grower gets the benefit of adapting to life without methyl bromide before the complete ban takes place. Growers contribute to the research landscape by allowing testing of alternatives under typical growing situations in a wide range of conditions, such as different soils, temperatures, and pest loads. These collaborations also "allow researchers to understand the constraints growers are working under," says Thomas J. Trout, research leader at ARS' Water Management Research Laboratory in Fresno, California.

Methyl Bromide: A Dream Chemical

For years, methyl bromide has been the magic bullet, a fumigant that almost completely eradicates pests and weed seeds in soil in preparation for planting. Unfortunately, it has also been identified as a chemical that depletes the earth's ozone layer, and thus its use is being phased out. It is scheduled to be completely out of use for soil fumigation in the United States in 2005.

This, of course, leaves growers without a chemical that can do it all. To survive, "growers will have to rely on pest management programs that are biologically knowledge-intensive," says ARS' Daniel O. Chellemi, plant pathologist, at the U.S. Horticultural Research Laboratory in Ft. Pierce, Florida.

To make the best use of any alternative, growers must not only be aware of the weeds, parasitic nematodes, and plant diseases present in their fields, but they must also have an understanding of the life cycles of each. According to Chellemi, "all methyl

bromide alternatives have an Achilles heel, so knowledge of pest biology will prove invaluable in selecting the best combination of alternatives and optimizing their performance."

What's Next?

Researchers actively maintain ongoing relationships with the growers in their geographical areas. By knowing the intimate details of the soil, weed, and pest types and the proposed alternatives, researchers can effectively approach the problems these growers face. For example, Chellemi maintains contact with growers in Fort Pierce, Florida, and encourages growers not only to test research alternatives, but also to try their own ideas.

One grower in the Fort Pierce area was applying Telone C35 to his tomato and pepper crops with some effectiveness, but he wasn't satisfied with the results. So, he asked Chellemi for a detailed explanation of its chemical properties. From this description and his past farming experience, the grower designed and manufactured a machine to apply the chemical to his crop area, resulting in better chemical efficiency.

Growers who participate in research trials make definite contributions. Obviously, they must commit plots for the research. But they also make labor available to harvest fields, provide equipment, and collect large blocks of data. Participating growers "make the philosophical commitment necessary to ensure they do the best job they can and collect the best data," says Noling.

Of course, using field plots for research trials is not without some risk, but the risk is fairly minimal. By the time a treatment reaches the field trial stage, it's been proven to have some effectiveness. The remaining question is how effective the alternative treatment is compared to methyl bromide.

In order to survive the methyl bromide phaseout, growers must make changes in the way they manage weeds and pests, incurring some risk. Smaller farmers generally can accept a small reduction in production, and those who are good managers will incur less crop loss. "Organization and good management practices will be more important than size," says Noling.

Growers involved in the research for methyl bromide alternatives are trying save their livelihoods. "Methyl bromide leveled the playing field," says Chellemi. "My job is trying to find a long-lasting, durable approach to pest control that is practical, efficacious and allows the farmer to profitably grow crops in a more environmentally acceptable production system."

USDA, Canada Collaborate on *Fusarium* Wilt

Family warfare has broken out in the *Fusarium* clan. While some members of this fungal family are beneficial, others are harmful. Researchers may soon prove that when it comes to *Fusarium*, like family, you can't live with them—or without them.

A pathogenic strain of *Fusarium oxysporum*, causes *Fusarium* wilt, a disease that afflicts many crops such as watermelon, muskmelon, and basil but is a bigger problem for tomato growers. Methyl bromide was used to keep this pathogen at bay, but now that methyl bromide is being phased out due to concerns about ozone depletion, other alternatives are under investigation.

Researchers at USDA and Canada's equivalent—Agriculture and Agri-Food Canada (AAFC)— are collaborating on studies that pit *Fusarium* species against one another. Pathogenic *F. oxysporum* was controlled with varying degrees of success by several harmless members of the *Fusarium* family.

Deborah R. Fravel, a plant pathologist at ARS' Biocontrol of Plant Disease Laboratory in Beltsville, Maryland, and George Lazarovits, a research scientist and team leader at the Southern Crop Protection and Food Research Center in Ontario, Canada, are working together to find a workable biocontrol solution. Biocontrol is the use of one organism to control another.

"The Beltsville group under Dr. Fravel's guidance was heading in a parallel direction with our work, and collaboration was obvious," Lazarovits says.

Just to get to this point, however, took plenty of groundwork. Fravel was sent to Florida to obtain soil samples from tomato fields. "We screened 450 microbes and found another *Fusarium* that helped control wilt," explains Dr. Fravel.

Into the Fields

In 1997, field plots were set up at the USDA Beltsville Agricultural Research Center to test selected biocontrol agents for control of *Fusarium* wilt in tomatoes. The agents chosen reduced *Fusarium* wilt in greenhouse tests: isolates of *F. oxysporum* (CS–20) and *F. solani* (CS–1), and a commercial biocontrol agent, SoilGard, containing *Gliocladium virens* strain G1–21. These were tested alone and in various combinations. In 1998, another combination treatment, consisting of a fungus (*G. virens* strain G1–3) and a bacterium (*Burkholderia vietnamiensis* strain Bc–F), was also tested.

Tomato plants were grown in soilless-mix plug trays (98 cells/tray) in the greenhouse for about 6 weeks before transplanting to the field. Beneficial *Fusarium* isolates were placed in the plug trays as liquid inoculum (5 ml of 106 colony-forming units/ml of suspension/cell) at the time of seeding and again 1 week before transplanting. SoilGard granules were worked into the soilless mix at a rate of 2 g/L before seeding. The combination of dry fungus and bacterium was applied

with a sticker as a seed treatment before planting and as a liquid 1 week before transplanting.

Plots were set up as single rows on 1.5-m centers, with 24 plants per 15-m row in 1997 and 12 plants per 7.6-m row in 1998. Pathogen inoculum of a race 1 isolate of *F. oxysporum* f. sp. *lycopersci* was incorporated into rows (2 kg/row of liquid culture) 1 day prior to transplanting in both years. In 1998, an additional 100 ml of inoculum was added to each transplant hole at the time of planting. Tomatoes were transplanted by hand in the fields, and overhead irrigation was used when necessary throughout the growing season. At the end of the season, stem sections from all plants were taken. Stem surfaces was sterilized and placed in agar plates on a *Fusarium*-selective medium to determine the incidence of plants systemically infected with the pathogen.

In 1997, very little disease developed, and none of the treatments showed any differences. Because no disease was noted, yield data were not taken that year.

But in 1998, biocontrol treatments containing *F. oxysporum* isolate CS–20 (CS–20, CS–1 + CS–20, and SG+CS–20) and the fungus plus bacterium treatment (G+B) significantly reduced the incidence of disease. Of the treatments, only CS–20 and G+B treatments showed significant effects on yield, with increased total weight, number of fruits, and average weight/fruit, compared with a pathogen control. The CS–20 and G+B treatments resulted in increased total weight of 34.3 percent and 37.7 percent, respectively, over the pathogen control. SoilGard and CS–1 alone showed no significant effect on disease incidence or yield.

The study shows that beneficial *Fusarium* strains can reduce tomato wilt and increase yield. Now researchers must figure out how the mechanisms of biocontrol work. Some beneficial strains work by competing with the pathogenic strains for nutrients and space. CS–20 seems to pump up the tomato plants' natural defenses against pathogens, a reaction called "induced systemic resistance."

Back to the Lab

ARS is testing several species of *Fusarium* for effectiveness in controlling *Fusarium* wilt, but one *Fusarium* cannot be easily distinguished from another. "We developed genetically tagged *Fusarium* isolates that can be readily tracked in the soil and on the plant," says Lazarovits. "The tags allow us to quantitatively recover the organisms from the environment they are introduced into."

Dr. Jian Bao, a molecular biologist who previously worked with Dr. Lazarovits and now works with Dr. Fravel, has provided data and beneficial strains of *Fusarium*—research tools Dr. Fravel's lab would otherwise have had to produce and work that could have taken a significant amount of research time.

Bao has developed a set of flourescent genetic tags for the beneficial *Fusarium* strains and another for the pathogenic strains. He will use these tags to determine where each strain resides in the plant.

Fravel asserts the collaboration is working well. "The labs have different strengths that complement each other," says Dr. Fravel. "Dr. Lazarovits' lab has done a lot of work in the molecular biology area. Our lab didn't have that level of expertise readily available."

Fravel continues, "ARS' strength is its extensive research in the biocontrol agents' mechanisms of action and the inoculum studies it's conducted."

"Most researchers have concentrated mainly on screening organisms for efficacy," says Lazarovits. "We need to do more on developing the tools needed to evaluate what happens to the organism in the environment we place it into."

Future Research

Using genetic tags, Fravel hopes to determine where the various *Fusarium* strains in one plant reside. "Researchers have worked on the supposition that beneficial *Fusarium* resides in the root," says Fravel. "Unfortunately, no one really looked."

Finding DNA fingerprints for all of the 350 *Fusarium* isolates found in the soil samples is a future goal, according to Fravel. This would enable researchers to quickly and accurately distinguish the good strains from the bad.

From East to West: The Asian Longhorned Beetle Has Landed

The Asian longhorned beetle has been causing quite a commotion in New York City and Chicago, since its 1996 arrival in the United States. Since then, it has spread around those two cities, leaving weakened trees that must be removed, chipped, and burned to prevent more widespread infestations.

As a result of the invasion, as of December 1998, the United States no longer accepts imports from China, including Hong Kong, packed in untreated wooden packing materials. These materials must be fumigated or treated with heat or preservatives prior to arrival in the U.S. Inspections at ports of entry, by USDA Animal and Plant Health Inspection Service (APHIS) inspectors, have been stepped up. An interim rule has taken effect, requiring treatment of solid wood packing materials to eradicate the beetle and any larvae. Shipments lacking certification of such treatments are subject to APHIS inspection for pest insects or re-export. Importers may be given the opportunity to separate the cargo from the packing materials at a location and in a time frame the inspector specifies. The solid wood packing materials must then be re-exported or destroyed, as directed by the inspector.

The Culprit

The Asian longhorned beetle can be a formidable adversary. As ARS entomologist Michael Smith says, "If its geographic distribution in China is any indication, it has the ability to live and thrive in a wide range of climatic conditions where suitable hosts abound." Indeed, the beetle seems to tolerate the cold winter weather and hot summer temperatures well, all the while feasting on various hardwood trees but especially maples, elms, poplars, willows, ashes and horsechestnuts. "If the relative proportion of trees found infested by the beetle in the United States thus far is an indication of its host preference, as opposed to merely a reflection of the relative species abundance in infested landscapes, it may have a distinct taste for maple trees, which for the eastern U.S. is particularly alarming," warns Smith. Cherry and various fruit trees are also at risk.

In New York and Chicago, street and park ornamental trees such as the Norway maple have taken the brunt of the beetles' invasion. These trees were planted because they show the ability to survive the assault of urban pollution and limited space. But now, the Asian longhorned beetle has compromised their survival.

During the summer months, the adult females lay eggs under the tree bark, leaving a round or oval wound on the trunk and branches. The larvae feed briefly under the bark and then burrow into the center of the tree to feed. When the larvae finally mature to the adult stage, they tunnel their way out of the tree, again leaving a wound in the trunk, this time a 3/8-inch hole. The internal damage sustained by the tree renders it vulnerable to secondary attack by other insects or diseases.

Economic Issues

The economic fallout has been significant: management of the beetles' initial 1996 New York infestation cost the state and federal governments more than $4 million. Last year, U.S. Department of Agriculture Secretary Dan Glickman signed a declaration of emergency, transferring $5.5 million in funds to aid in the detection of the Asian longhorned beetle, identification of infested areas, control and prevention of the beetle's spread to noninfested areas, and eradication of the pest.

"The Asian longhorned beetle is a pest that threatens trees in our forests, rural areas, and even urban neighborhoods," Glickman said. "This additional investment will enable us to further expand our priority prevention and control efforts."

The beetle threatens not only urban economies, but agricultural economies as well. The sugar maple, from which maple syrup is derived, is a particular favorite of the Asian longhorned beetle. Maple syrup production in this country generates almost $9 million in revenue each year. This is but a drop in the bucket to the overall loss potential; total annual revenue from all industries that would be affected by a widespread Asian longhorned beetle infestation in New York alone tops $11 billion. If this beetle and other wood-boring pests were to become fully established in the United States, they could damage industries that generate combined annual revenues of $138 billion.

Interventions

Scientists are currently attempting to find workable strategies to control the Asian longhorned beetle infestation. "The beetle spends the vast majority of its life cycle within the tree, specifically within the interior wood," says Smith. "This makes it particularly difficult to kill after the tree is attacked."

APHIS scientists have conducted field trials in China for 2 to 3 years and are now proposing systemic insecticide treatments by various injection methods, either by injection into the soil or injection directly into the lower tree trunk just above the roots of infested trees. Theoretically, the injections facilitate transport of the pesticide's active ingredient from the application site (root zones) to active areas of tree growth, where the adult beetles feed and lay eggs.

However, until such methods of eradication are realized, the only option remaining in infestation areas is to remove the infested trees, put them through a chipper, and burn the chips. This combination of chipping and burning the wood remains the most effective and economical method of destroying the beetle larvae and eggs within the trees. If this pest is to be eradicated, it is clear that U.S. borders must be guarded vigilantly.

The Interim Rule and Methyl Bromide

Under APHIS' interim rule, several treatment methods have been approved: application of preservatives, heat treatment, and fumigation. Methyl bromide is the only approved substance for fumigation of the wood packing materials.

Methyl bromide used to treat quarantine pests such as the Asian longhorned beetle is exempt from the phaseout. However, methyl bromide may become unavailable at some point in the future because the quarantine exemption may be removed or manufacturers may lose interest in producing it for the small quarantine market. To avoid having to rely on only one fumigant, ARS scientists, at the Horticultural Crops Research Laboratory, in Fresno, California, are testing several potential postharvest fumigants as alternatives to methyl bromide. (See the technical report that follows on the sulfuryl fluoride research at Fresno.)

At the moment, only the treatment of solid wood packing materials prior to arrival on U.S. shores—and the program of destroying infested trees—stand in the way of a widespread and economically devastating infestation of the Asian longhorned beetle.

Technical Reports

Sulfuryl Fluoride: A Disinfestation Treatment for Walnuts and Almonds

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In California, the codling moth, *Cydia pomonella* (Linnaeus), is the major insect pest of walnuts while the navel orangeworm, *Amyelois transitella* (Walker), is the major insect pest of almonds. The navel orangeworm is also a major pest of walnuts, particularly if populations are allowed to increase in orchards where nuts have already been damaged by the codling moth. Although these field pests are carried into the storage environment with harvested commodities, they do not thrive in storage and subsequently perish. However, their presence in the post harvest environment, albeit short lived, presents a problem for some export markets because both insects are considered quarantine pests by some countries. In addition, the European Union requires imported walnuts to be fumigated with methyl bromide to eliminate the codling moth and navel orangeworm to meet phytosanitary requirements.

Sulfuryl fluoride (=Vikane®) has been identified as a potential replacement for several methyl bromide uses. It has been registered and labeled in many parts of the world as a structural fumigant for 40 years but has no food tolerance. Its insect efficacy is comparable to that of methyl bromide against the active life stages of many insects. The objective of this study was to evaluate the toxicity of sulfuryl fluoride to codling moth and navel orangeworm to determine if it could be used to replace methyl bromide as a treatment for nuts infested with codling moth and navel orangeworm.

Naked life stages of the codling moth and the navel orangeworm contained in glass dishes or vials were exposed to sulfuryl fluoride inside 28.3-liter Labconco® vacuum desiccators. For codling moth, last instars (2 wk in diapause) were tested as well as aged (1, 2, or 3 days old) eggs. For navel orangeworm, last instars and similarly aged eggs were used. The insects were taken from laboratory cultures reared at 27°C, 60% RH, and a 12:12 (L:D) photoperiod. Diapausing codling moth larvae were reared under diapause-inducing conditions at 18 ± 0.5°C, 60% RH, and an 8:16 (L:D) photoperiod. Tests were conducted at normal atmospheric pressure (NAP) and at 100 mm Hg pressure (VAC). Fumigant concentrations were determined by gas chromatography as described by Zettler et al. (in press). Exposure times were 2 and 4 hours at 20 and 25°C. All mortality data were analyzed by probit analysis. Based on the LC_{99}, the CxT was calculated for each life stage tested.

Walnuts artificially infested with diapausing larvae of the codling moth were exposed to sulfuryl fluoride. Larvae were individually placed into walnuts through a hole previously drilled through the shell. After inserting the larva, the hole was sealed. Infested nuts along with non-infested walnuts were allowed to equilibrate at 15.6°C overnight. On the day of the fumigation, fifty infested nuts were placed in a loose-mesh bag, admixed with 6.8 Kg of non-infested nuts and placed in each chamber to produce a chamber load of 33%. Chambers were equipped with an internal fan for recirculating the chamber atmosphere during fumigation. Each dose was replicated at least three times, each on a different day. Exposure times were 24 hours at normal atmospheric (NAP) followed by forced aeration for 2 hours at a rate of 3.4 to 3.6 liters per minute and then 22 hours of passive aeration with the chamber doors open. Following aeration, infested nuts were stored at 15.6°C for 7 days at which time insect counts were made.

Sulfuryl fluoride was toxic to diapausing codling moth larvae at relatively low dosages. The LC_{99} CxT product was 127 mg hours/liter (63.6 mg/liter in 2 hour fumigation). VAC fumigation reduced the CxT product by about half to 68 mg hours/liter (34 mg/liter in 2 hour fumigation). Tebbets et al. (1986) also found that, when compared with NAP fumigation, VAC fumigation reduced LC values of this insect by half when fumigated with MB. On the other hand, eggs were relatively tolerant to sulfuryl fluoride fumigation. The tolerance differed with age (3-day > 2-day > 1 day). Whereas VAC fumigation decreased CxT values for larvae, it had no significant effect in reducing tolerance of eggs of any age. In fact, VAC fumigation was antagonistic to 1 and 2 day-old eggs resulting in CxT values higher than those for NAP fumigation.

Similar results are shown for navel orangeworm. Based on the CxT products, sulfuryl fluoride was about equally toxic to non-diapausing 5th instars of navel orangeworm as to diapausing larvae of codling moth. However, VAC fumigation had more effect on increasing sulfuryl fluoride susceptibility of navel orangeworm than of codling moth. Four-hour VAC fumigation reduced the NAP dose of 35.3 mg/L (CxT = 141 mg hours/liter) by more than 80% to 6.6 mg/liter (CxT = 26 mg hours/liter). Navel orangeworm eggs were more tolerant to sulfuryl fluoride than were codling moth eggs. Like codling moth eggs, navel orangeworm eggs showed varying tolerance to the fumigant and the tolerance was age dependent. VAC had no effect in reducing the CxT values except for 3 day old eggs, the most tolerant age for eggs of both species.

The present fumigation schedule for disinfesting walnuts infested with diapausing codling moth involves 24 hour fumigation at NAP at a dose of 56 mg/liter methyl bromide, the minimum effective dose (MED) required for complete control. Results of confirmatory tests showed that the MED for sulfuryl fluoride under the same conditions was 8 mg/liter. Thus, sulfuryl fluoride was 7X more toxic than methyl bromide. Sorption of sulfuryl fluoride by the walnuts was

only 30% compared with 80% for methyl bromide. Low sorption is indicative of rapid penetration into and aeration out of a commodity and thus reflects reduced chemical residues on the commodity.
When walnuts and almonds are harvested, first to fifth instars of codling moth and navel orangeworm are present from overlapping broods in the orchards. These larvae can freely infest the nuts while on the tree. Only rarely, however, does the codling moth remain in walnuts. When it does, in late August and early September, the fifth instars may enter diapause. This is a very rare occurrence because most larvae destined for diapause leave the nut. The fumigation treatment approved by Japan for these insects in walnuts is aimed at the diapausing larvae of codling moth because this is the most tolerant life stage that could infest the nut at harvest. The eggs don't occur naturally on walnuts or almonds at the time of harvest. Thus, based on larval toxicity, low sorption by the walnuts, and the fact that only the larvae are present in exported nuts, sulfuryl fluoride appears to be a viable replacement for methyl bromide under these conditions.

Although a 24-hour fumigation is not commercially practical for disinfesting walnuts being shipped to the European Union or Japan, the increased toxicity of sulfuryl fluoride under VAC conditions indicates the exposure period could be shortened to as little as 4 hours and still produce a CxT product that would produce complete control.

Insect Management in Food Processing Facilities with Heat and Diatomaceous Earth

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With the impending loss of methyl bromide, the use of high temperature as an insect control tool in food processing facilities is being revisited. This involves heating all or part of a facility to 120–140°F (49–60°C) for 20–36 hours. Some facilities in North America have successfully used heat treatments for more than 75 years to manage insect pest populations while this is a completely new concept for most processors. Heat kills insects in two ways: denaturing proteins inside the insect's body and disrupting the waxy layers on the outside of the insect causing dehydration. All life stages of stored-product insects will die at these temperatures in less than one hour, but the additional time is required for the heat to penetrate into processing equipment and the building itself. Not all facilities are well suited for heat treatment; wooden structural elements and equipment may dry out, warp or crack during heat treatment and some plastic materials used in modern processing equipment may warp or become brittle. Additionally, without good ventilation heat is generally not well distributed within a room during a heat treatment resulting in areas that are under-heated

A collaborative research project between the U.S. Department of Agriculture and Agriculture & AgriFood Canada is examining the use of diatomaceous earth in combination with heat for insect management in food processing facilities. Diatomaceous earth is the skeletal remains of single-celled algae, or diatoms, that formed thick layers in ancient seabeds when they died. Diatomaceous earth is comprised predominantly of silicon dioxide and is a fine, crumbly substance used in insulating materials, abrasives, ceramics, filters, as a food additive in toothpaste and as an anti-caking agent in artificial sweeteners. Diatomaceous earth has low mammalian toxicity and is registered as a feed additive and also is used as a natural insecticide. Mortality in insects is not immediate, as expected with traditional chemical insecticides, but will occur after a period of time. When diatomaceous earth is combined with heating, there can be synergism that causes insects to die more quickly and at lower temperatures than when insects are exposed to diatomaceous earth or heat alone.

Field trials of the combined treatment have been conducted in cereal processing plants in Peterborough, Ontario, and Manhattan, Kansas. The objective of both studies was to examine the combined impact of high temperature and diatomaceous earth on the mortality of adult confused flour beetles, *Tribolium confusum*, in a flour mill environment during heat treatment. The confused flour beetle was selected for the tests because of its high tolerance to diatomaceous earth and is a common pest of food processing facilities. Protect-It™ (Hedley Technologies, Mississauga, Ontario), an enhanced diatomaceous earth formulation was used in both studies and applied as a dry powder with an electric duster at a rate of about 6.5 oz/1000 ft2 (2 g/m2). The duster created a cloud of diatomaceous earth that settled to the floor in about 2 hours. Prior to the diatomaceous earth application, plastic sheets were taped to the floor to mask out the insecticidal dust in areas to serve as undusted controls. Plastic rings were glued to the floor using a removable sealant. The rings were placed on the floor to create diatomaceous earth-treated and untreated arenas. Adult confused flour beetles, 50 beetles per ring, were monitored at 60-minute intervals during the heat treatment and the dead insects were removed after each inspection. Steam heaters were used at both locations to reach and hold a target temperature of 122°F (50°C) for 24 hours.

Effectiveness of the diatomaceous earth and heat treatment was determined by the duration of survival during the treatment, the temperature at time of death, and the percentage of dead beetles at the end of the heat treatment. The beetles exposed to diatomaceous earth plus heat were all dead when temperatures reached

106°F (41°C) while those exposed to heat alone were dead when temperatures reached 115°F (46°C). These lower temperatures also translated into a shorter duration (13 hours) for the diatomaceous earth treatment compared to the heat alone (32 hours). The rate of heating also will impact the duration of heating and insect mortality. The researchers suspect that the insects will be more susceptible to a high rate of temperature increase that would minimize insect escape to cooler areas or adjusting to higher temperatures.

In areas that are difficult to heat to temperatures lethal to insects, an application of diatomaceous earth could be of value. Even if the insects are not killed during a combined diatomaceous earth plus heat treatment, the diatomaceous earth offers residual value for insect control if left in place after treatment. Because of the residual value and low mammalian toxicity of diatomaceous earth, it may be suitable for use in combination with heat in areas containing heat-sensitive equipment. It would also be useful in areas that are difficult to heat such as along outside walls, windows and basement floors.

Heat alone has already proven to be an effective and safe alternative to methyl bromide fumigation in facilities that can withstand the temperature requirements. Diatomaceous earth is also proven to be effective for controlling stored-product insects and can fill a niche where high temperature treatment may not be effective or practical.

Before there can be wide-spread acceptance of the combination treatment, there are four issues that need to be addressed. Resident insect populations often are in more protected locations, such as inside processing equipment, cracks and crevices in the floors and walls, and may never contact the diatomaceous earth or lethal temperatures. The researchers have demonstrated that heat penetrates quickly into flour milling equipment but currently there are no diatomaceous earth products labeled for use on food contact surfaces. Since diatomaceous earth is already used as a feed additive, approval for use in food processing equipment may be granted. Additionally, there remain questions about the abrasive characteristics of diatomaceous earth and what damage may result if used inside processing equipment. Given the low application rates, this is not likely to be a significant issue, but needs to be addressed. Finally, on a full plant basis, there are no data that conclusively prove that a combined treatment of diatomaceous earth and heat is better than either treatment individually. If the combination treatment does prove best, the question remains of how low the temperature can be dropped and still offer adequate insect control.

Depending on how these questions are answered, adopting heat treatments with diatomaceous earth for insect management will reduce the amount of traditional chemical insecticides used in the food processing industry. Additionally, it will further minimize worker safety issues associated with structural fumigation as well as the potential of toxins inadvertently contaminating food products.

Propylene Oxide: The Registered Fumigant, the Proven Insecticide as a Methyl Bromide Alternative

Tom Griffith & Morris Warren, ABERCO, INC.

This report is an attempt to demonstrate that the use of the New Old Fumigant, propylene oxide, can replace methyl bromide in many applications. With 40 years of food use history, we expect propylene oxide will satisfy all the tests of availability, effectiveness, safety and practicality. ABERCO, INC. has held the EPA Registration since 1984 and is now the sole registrant for the use of propylene oxide for fumigation purposes. The EPA label reads, "to aid in the control of microbiological spoilage and as an insecticidal fumigant for the control of stored product insects, to reduce bacterial and mold contamination in processed spices, cocoa and processed nutmeats (except peanuts)."

Worldwide, over ten billion pounds of propylene oxide are produced annually. Since propylene oxide is a basic chemical intermediate, and it is used to produce a wide variety of products such as urethane foams, cosmetics, polymers, starch modifiers, food emulsifiers and, of course, propylene glycol, it is designated as a GRAS (Generally Regarded as Safe) food additive. Propylene glycol is also the basis for the non-toxic Sierra anti-freeze. The hydrolysis of propylene oxide to propylene glycol will be explored later.

The total effectiveness of sterilization doses of propylene oxide against the pathogenic bacteria *Salmonella* and *E. coli* 157 in nuts, cocoa and spices has been well documented, while growers routinely treat their products with propylene oxide to reduce other bacteria, molds and yeast as well.

Industries use methyl bromide to control stored products insects. With the accelerated timetable for banning all production and importation of methyl bromide beginning January 1, 2001, our top priority was to demonstrate the effectiveness of propylene oxide in killing the insects found in cocoa beans, in-shell nuts and spices. This study is underway at the Dried Fruit Association of California. The early results are: propylene oxide, at doses similar to typical methyl bromide doses, kills all stages of the confused flour beetle, warehouse beetle, red flour beetle, cigarette beetle and the Indian meal moth. Propylene oxide is particularly effective on eggs. A study on the rate of kill on the Codling Moth larvae in walnuts, as well as, off gassing studies on raisins, prunes and figs are scheduled.

The allowable residue tolerance for propylene oxide is 300 ppm. However, these residues are not persistent as they will evaporate rapidly from the substrate due to the relatively high vapor pressure of propylene oxide. Furthermore, propylene oxide will

react in the human stomach to form (GRAS) propylene glycol. A research program was established utilizing radio labeled propylene oxide for 100% accuracy and irrefutability.

The test was conducted in two parts: 1) Using simulated stomach juice at pH 1.0, the PPO half life was 63 seconds. 2) Using human stomach juice at pH 1.5, the PPO half life was 111 seconds.

Notes: All PPO converted to (GRAS) propylene glycol. No propylene chlorohydrin, a suspected by-product, was found. These facts indicate that propylene oxide is not likely to be carcinogenic or harmful.

The conversion of propylene oxide to propylene glycol is catalyzed by both acid and base and the rate of reaction is determined by the concentration of the catalyst. This is also why propylene oxide will hydrolyze to propylene glycol in the soil. Both propylene oxide and propylene glycol are biodegradable.

The initial fumigation work shown above was done with flammable 100% PPO in vacuum chambers at ~80 degrees F for 4 hours @ 100mm Hg according to our label. Note, it is very important not to confuse propylene oxide with ethylene oxide because their structure is similar. Ethylene oxide may not be used on food products since it is a known carcinogen, whose by-products are also toxic. Furthermore, it is explosive at concentrations of 1% to 100% in air. Propylene oxide, on the other hand is flammable from 3% to 37% in air, much like propane. Since many of the new uses contemplated will be better served by a nonflammable insecticide, a collaborative study with the USDA at Fresno will determine the efficacy of a nonflammable mix of 8% PPO/ 92% CO_2 under atmospheric conditions. This mixture is currently on the EPA label. There is a history of using this mix at ambient atmospheric conditions in stack and structural fumigation, but the insecticidal efficacy data on the commodities in question needs to be confirmed. Delivery systems for this gas mixture can be tailored for the application. The inhalation limits for 100% propylene oxide are: OSHA–100 ppm and EPA–20 ppm. Worker safety issues are easily handled by the most basic precautions. Researchers at the University of Tennessee have determined that methyl bromide is 92.8% effective against *Aspergillus flavus* and *A. parciticus*, the molds required for the production of aflatoxin. Phosphine, however is reported to be only 7.1% effective at the same dose. Since propylene oxide is widely used to reduce mold and yeast in nutmeats and spices, the registrant has commissioned the Dried Fruit Association of California to determine the efficacy of propylene oxide against these two molds. The control of the target insects and these molds in the post-harvest environment will reduce the formation of aflatoxin in the commodity. This dual function will offer another portion of the advantages obtained by using methyl bromide.

There are references indicating that propylene oxide is a possible nematicide effective against both egg masses and larvae of *Meloidogyne hapla* in soil. Propylene oxide has been reported to be highly mobile in soil and has physical characteristics that appear to be ideal for soil fumigation. Certainly, its biodegradability and its conversion to non-toxic biodegradable propylene glycol in ground water answer some of the key environmental questions. ABERCO. INC. is planning some preliminary studies in this area.

Propylene oxide is already approved for use on food products and it passes the key tests of availability, effectiveness, safety and practicality. Although more information is needed to confirm its efficacy, it is readily apparent that propylene oxide is an important viable contender for replacing methyl bromide in some critical applications.

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